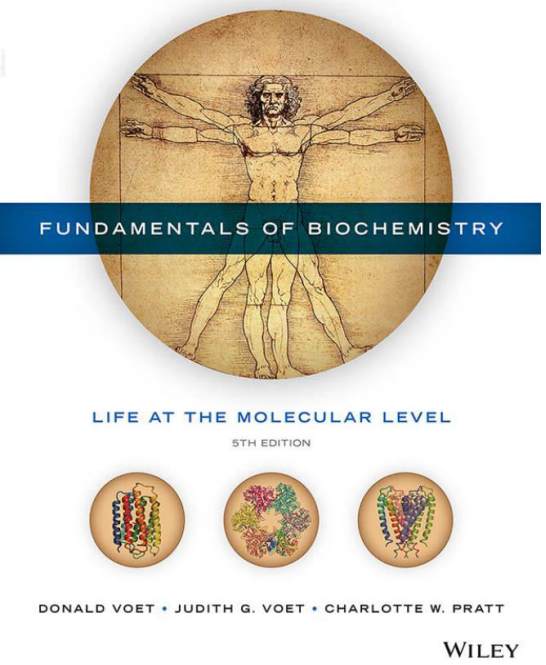
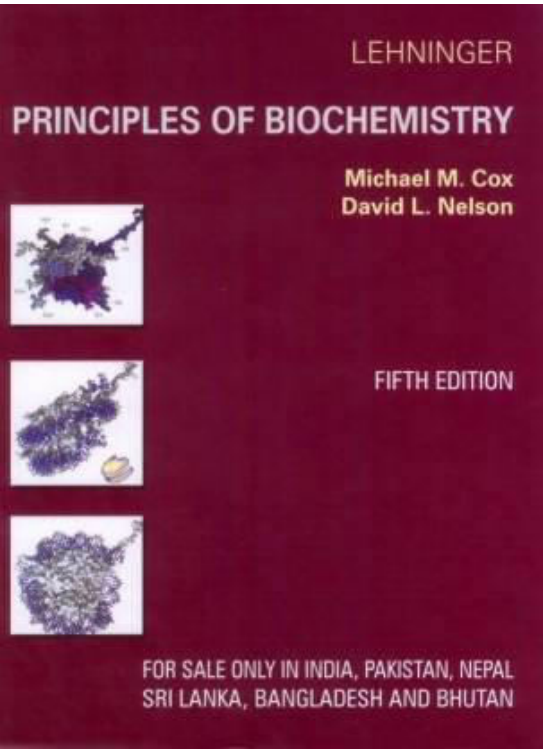


Irreversible Inhibitions



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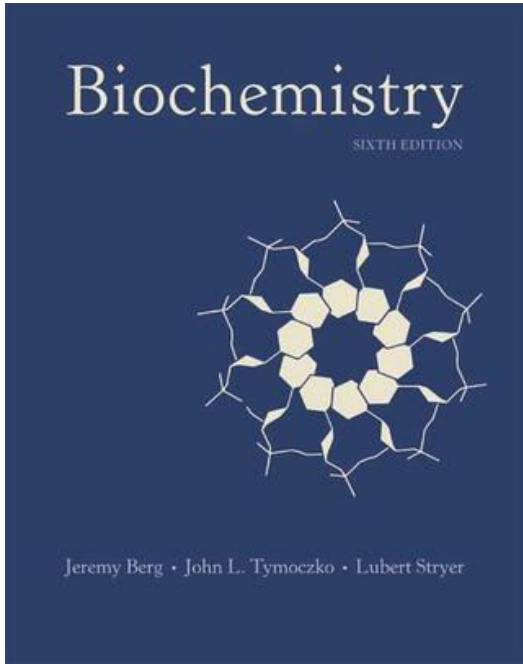


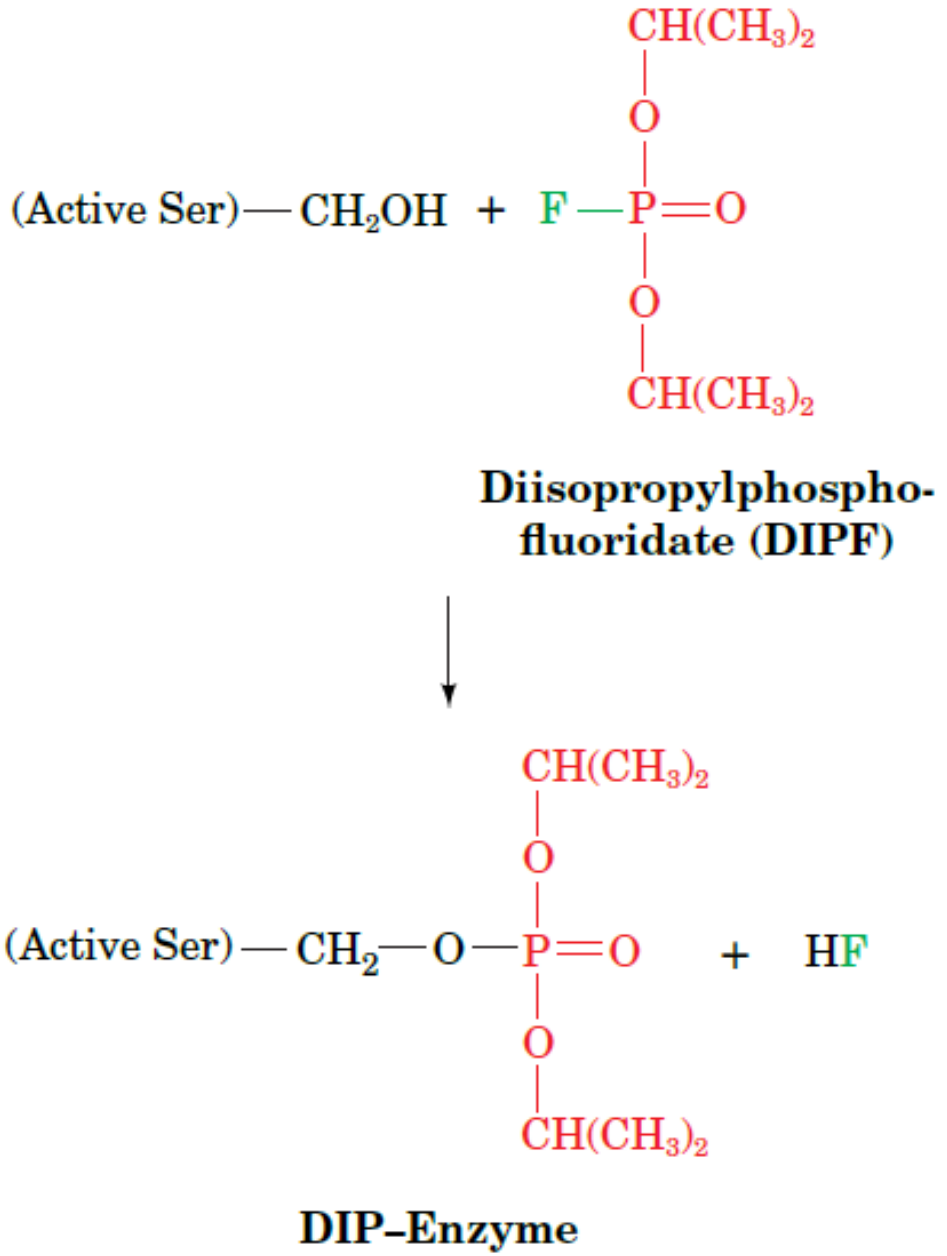
Inactivators or Irreversible enzyme inhibitors

Irreversible enzyme inhibitors, or inactivators, bind to the enzyme so tightly that they permanently block the enzyme's activity.

DIPF (Diisopropylphosphofluoridate)

- A diagnostic test for the presence of the active site Ser of serine proteases is its reaction with **DIPF** which **irreversibly inactivates the enzyme**.
- Other Ser residues, including those on the same protein, do not react with DIPF.
- DIPF reacts only with **Ser 195 of chymotrypsin**, thereby demonstrating that this residue is the enzyme's active site Ser.

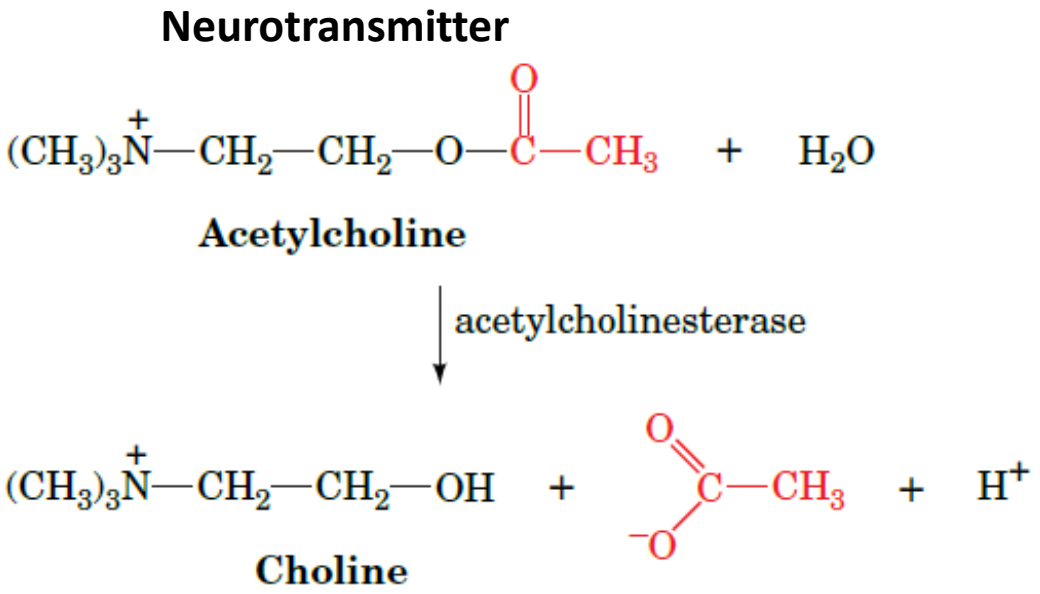
The reason that DIPF is such an effective inhibitor of serine proteases is because its **tetrahedral phosphate group** makes this compound a **transition state analog**.



Nerve Poisons

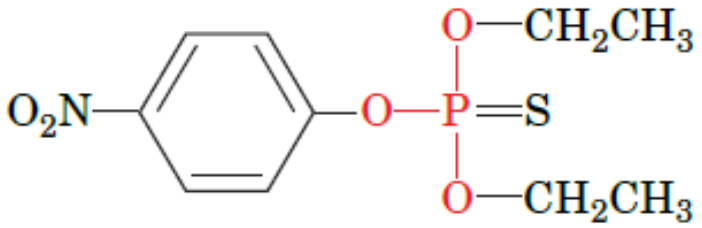
The use of **DIPF** as an enzyme-inactivating agent came about through the discovery that organophosphorus compounds such as DIPF are potent nerve poisons.

The **neurotoxicity of DIPF** arises from its ability to **inactivate acetylcholinesterase**, an enzyme that catalyzes the hydrolysis of acetylcholine:

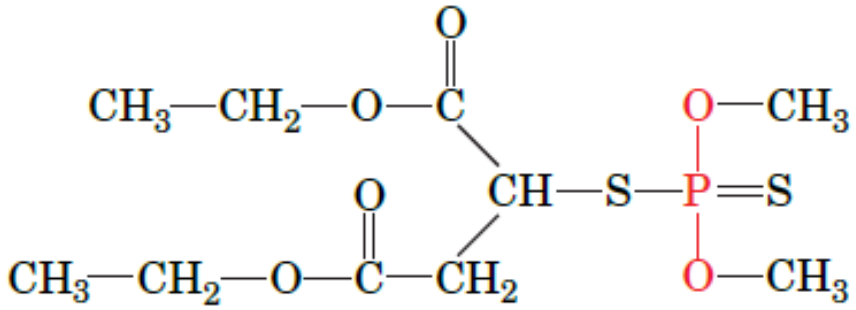


DIPF is so toxic to humans (death occurs through the inability to breathe) that it has been used militarily as a **nerve gas**.

Related compounds, such as **parathion** and **malathion**, are useful **insecticides** because they are far more toxic to insects than to mammals.



Parathion



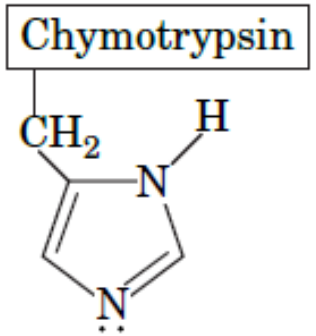
Malathion

TPCK

- A second catalytically important residue, **His 57**, was discovered through **affinity labeling**. In this technique, a **substrate analog bearing a reactive group specifically binds at the enzyme's active site**, where it reacts to form a stable covalent bond with a nearby susceptible group. **The affinity labeled group(s) can subsequently be isolated and identified**.

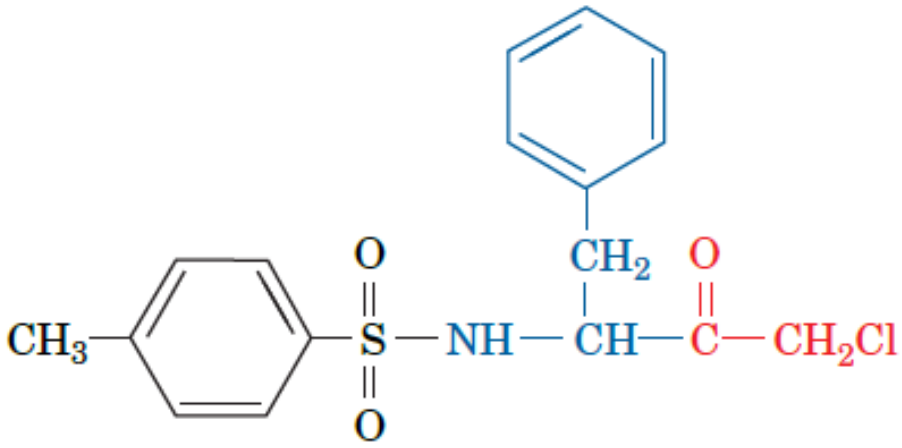
- **Chymotrypsin** specifically binds **tosyl-L-phenylalanine chloromethylketone (TPCK)** because of its resemblance to a **Phe residue**. Active site bound TPCK's chloromethylketone group is a **strong alkylating agent**; it reacts only with His 57, thereby inactivating the enzyme.

- **Trypsin**, which **prefers basic residues**, is similarly inactivated by TPCK.

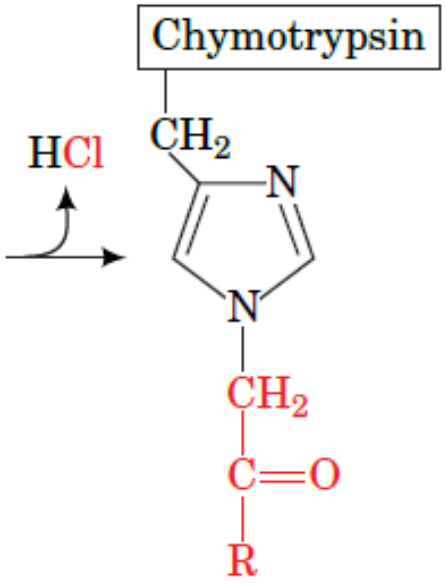


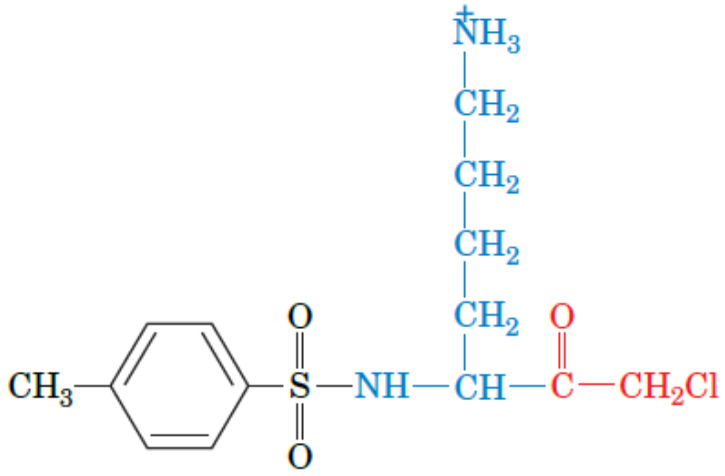
His 57

+



Tosyl-L-phenylalanine chloromethylketone (TPCK)

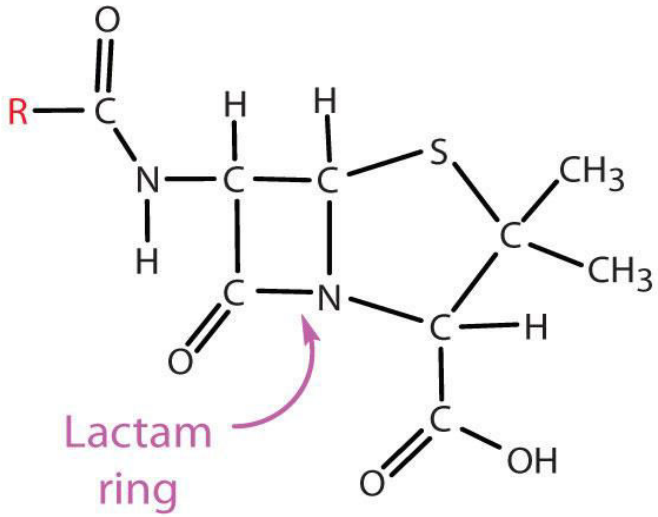
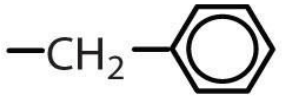
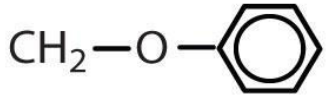
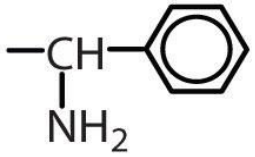
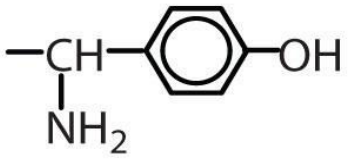
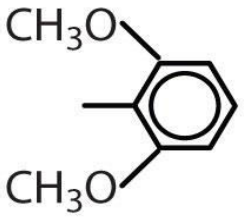


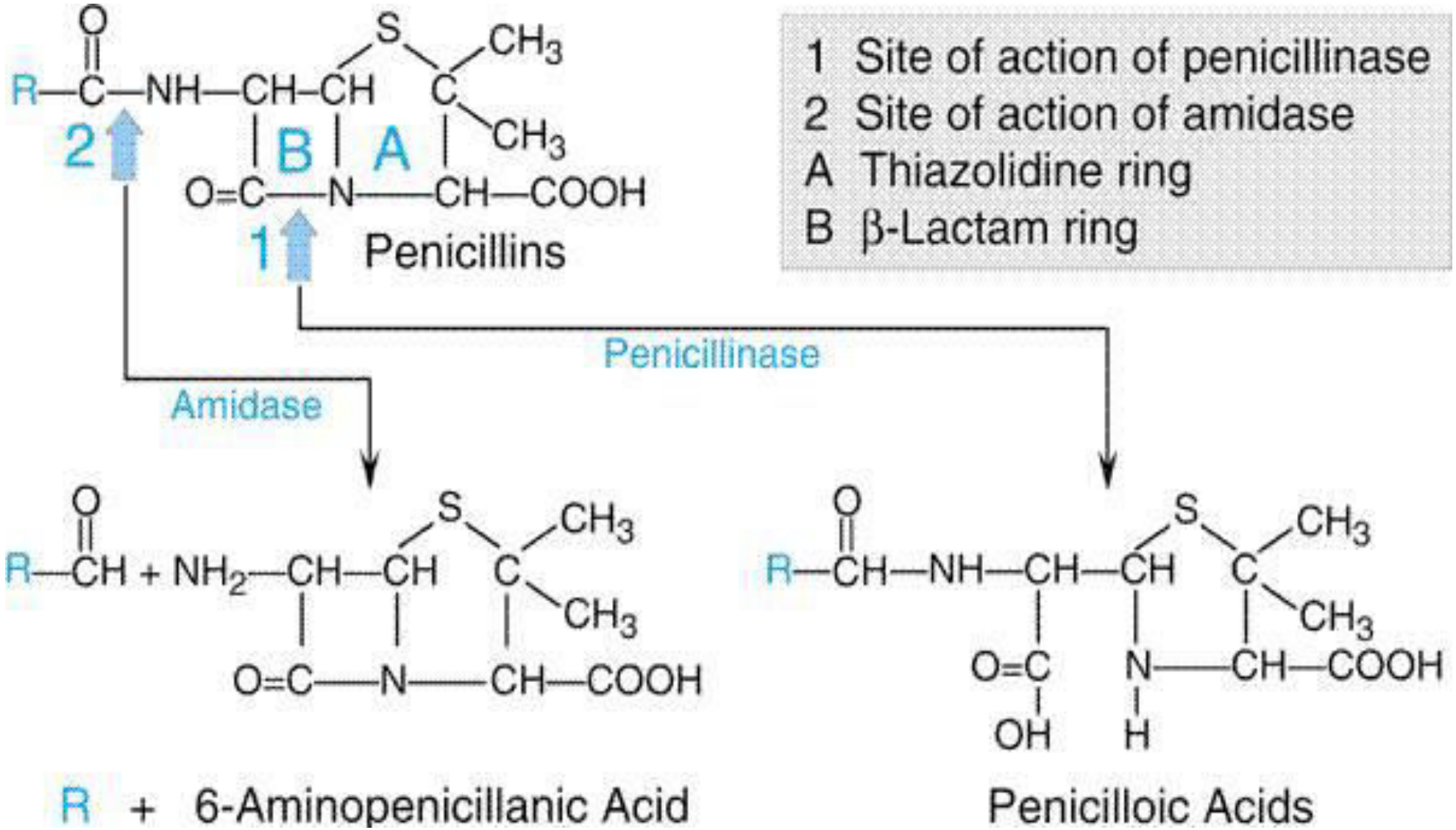


Tosyl-L-lysine chloromethylketone

Trypsin

Penicillin

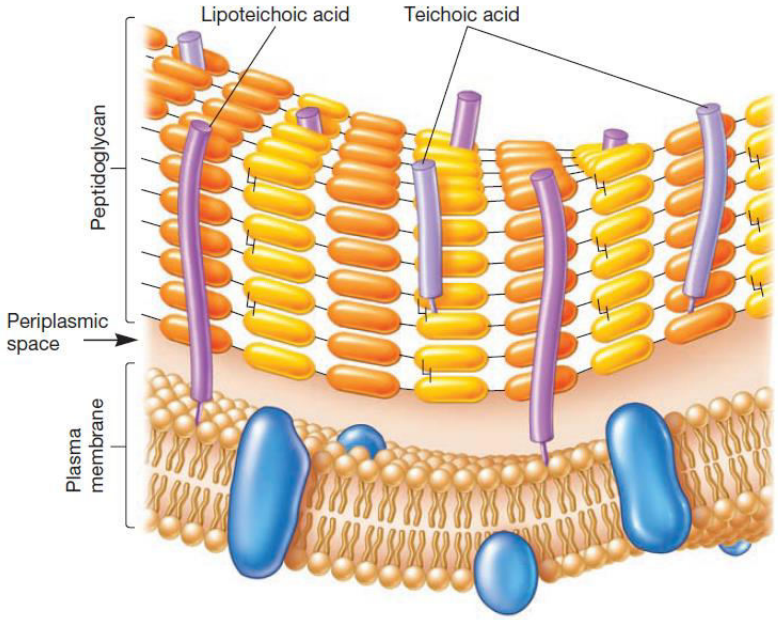
Penicillin Structure	R Group	Drug Name
		penicillin G
		penicillin V
		ampicillin
		amoxicillin
		methicillin

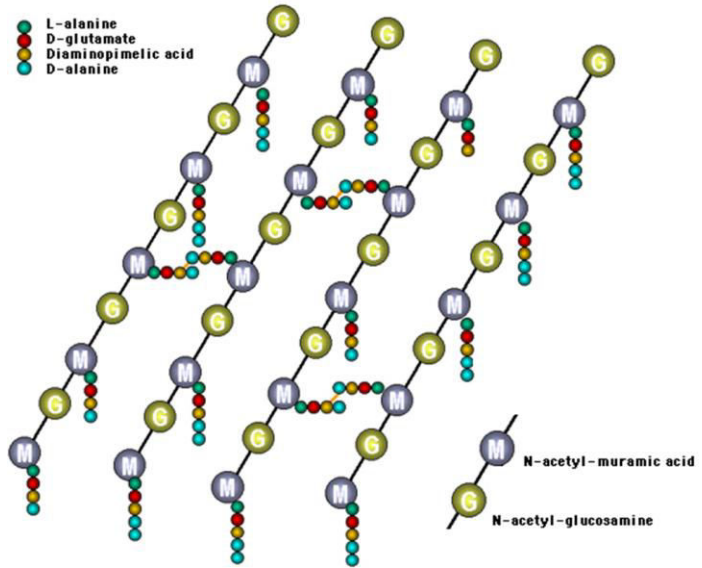


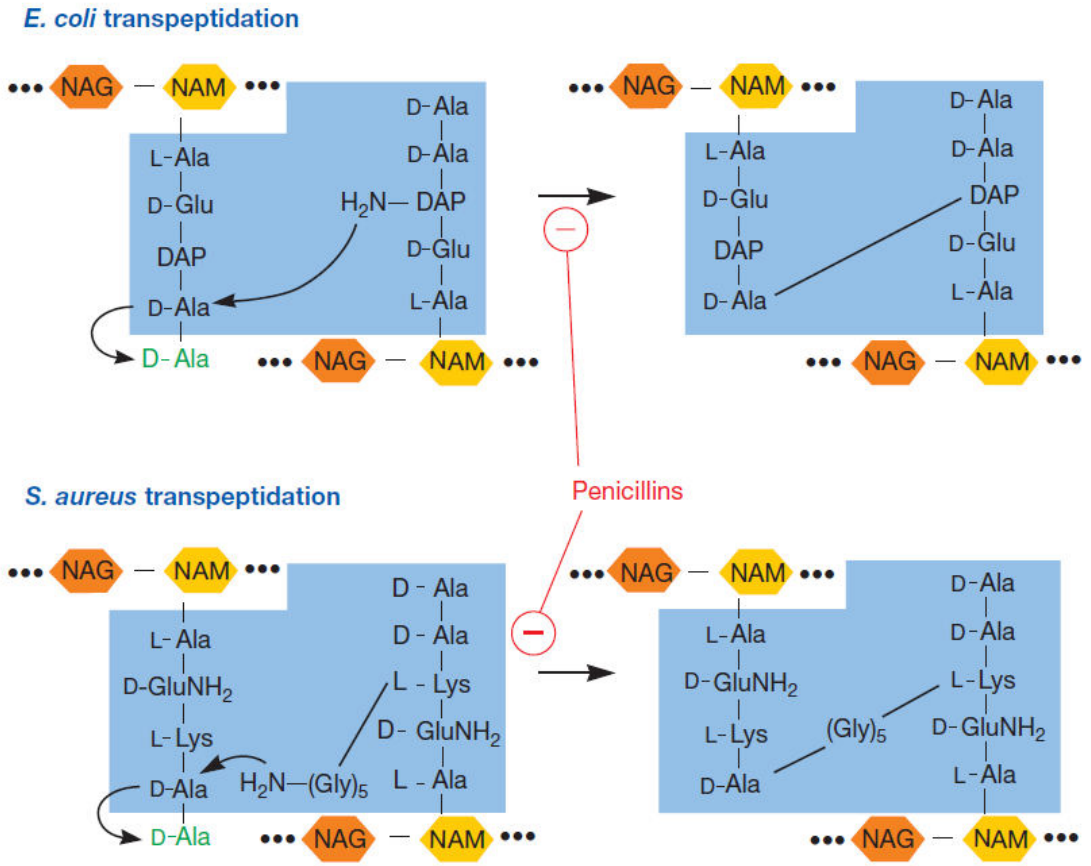
Active material Raw material for other penicillin

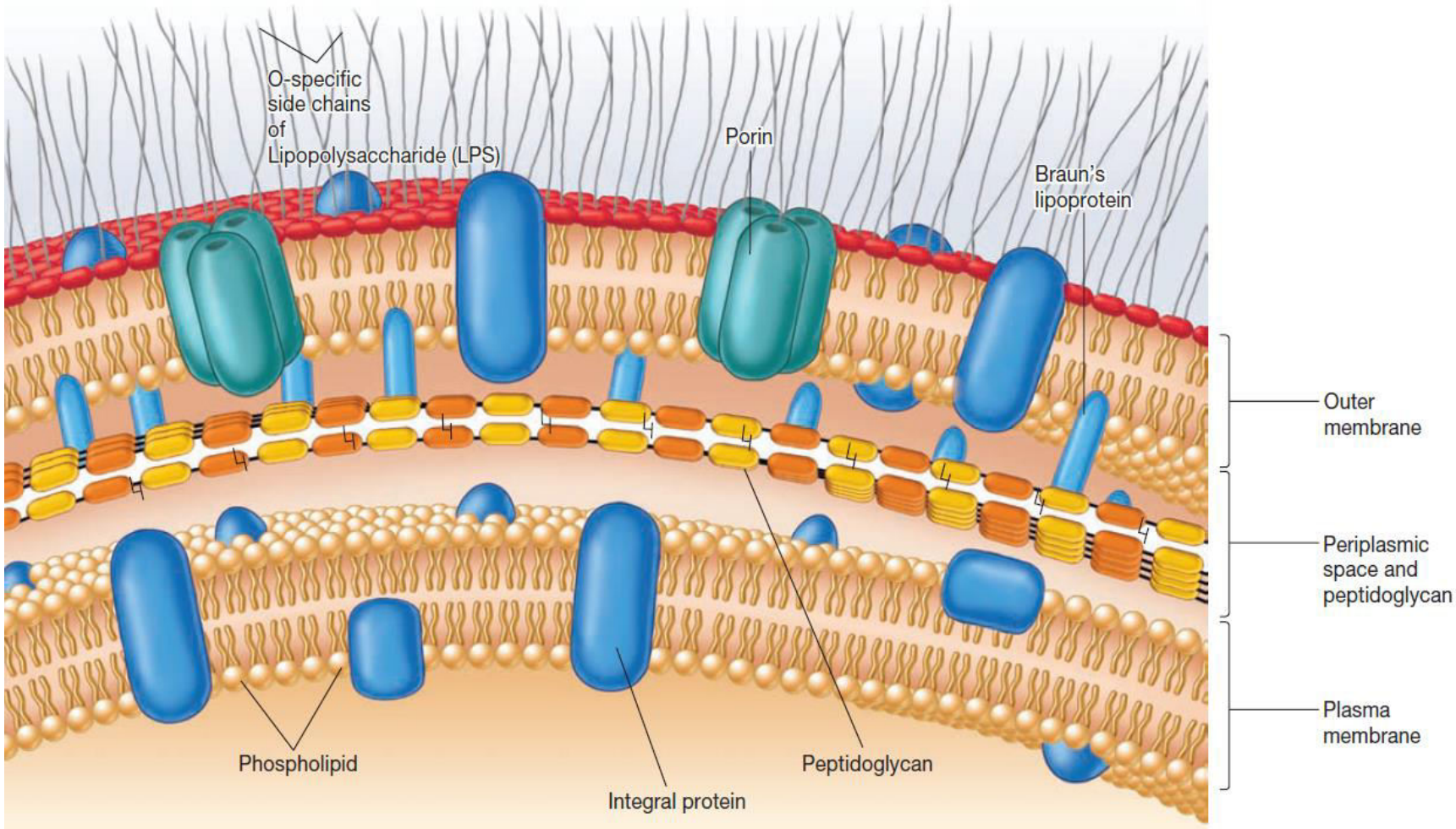
Inactive (Major Determinant) Responsible for hypersensitivity

Mechanism of Action

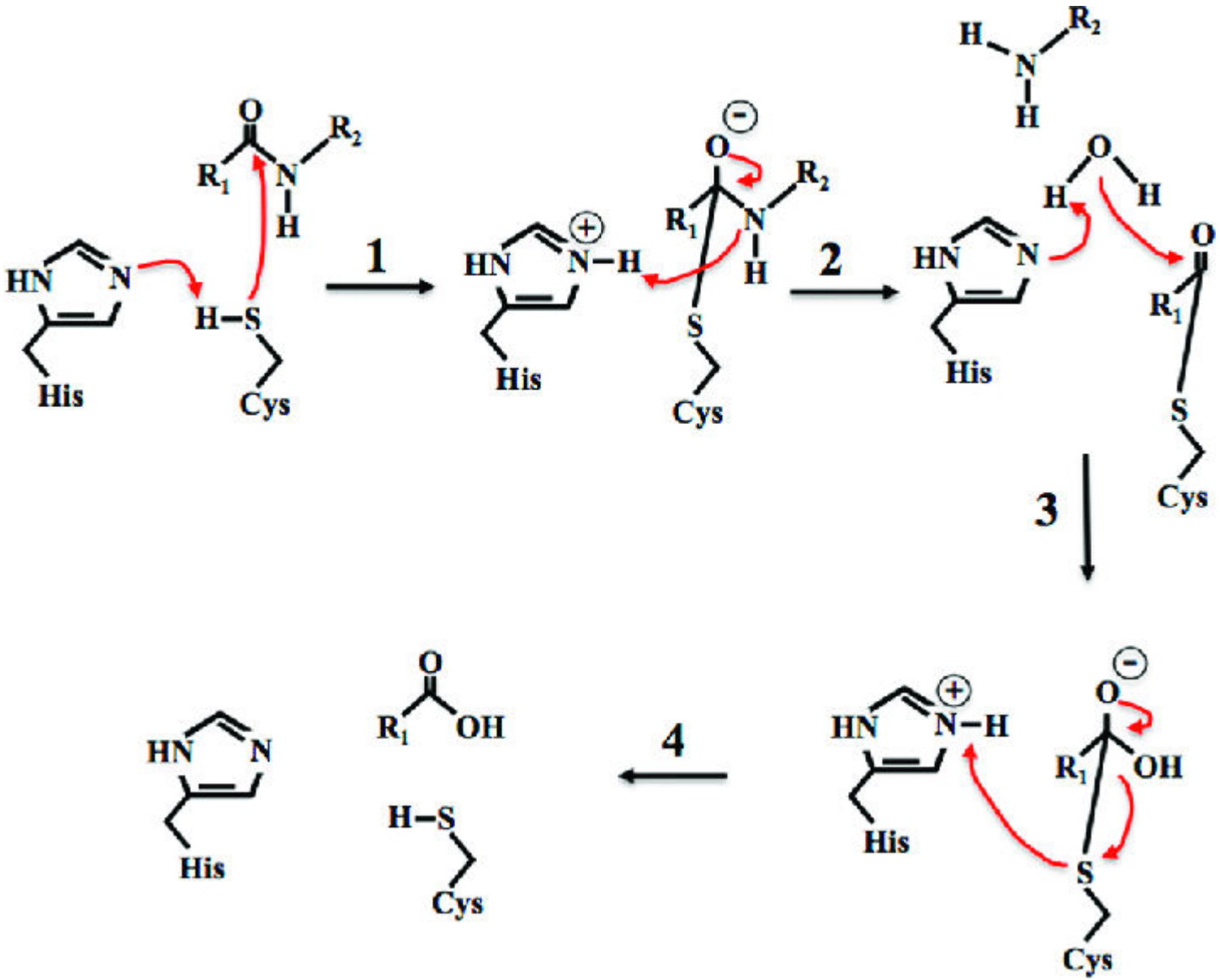


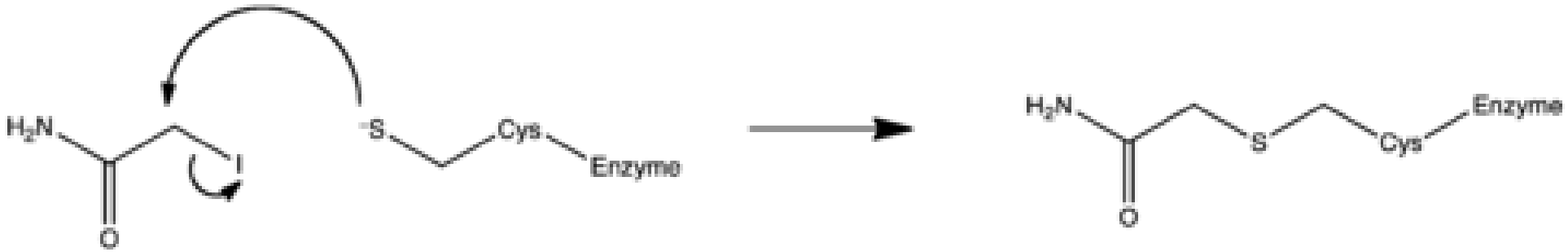





O-specific
side chains
of
Lipopolysaccharide (LPS)
Porin
Braun's
lipoprotein
Outer
membrane
Periplasmic
space and
peptidoglycan
Plasma
membrane
Phospholipid
Integral protein
Peptidoglycan

Iodoacetamide and cysteine proteases




H2N
O
I
S
Cys
Enzyme
H2N
O
S
Cys
Enzyme